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(54) Fluid analysis with particulate reagent suspension.

(57) An improvement in the analytical method for determining the presence or concentration of analyte in a solution comprising detecting a dissolved detectable substance in solution with a detecting means, for example, by passing a solution containing a detectable substance through a detection means for determining the presence of concentration of detectable substance in the solution. The improvement comprises mixing the solution with one or more particulate reagents to form a suspension, the particulate reagents modifying the solution to yield a concentration of total detectable substance which correlates with the concentration of original analyte, and passing the solution through the detecting means. The particles can be used to replace an analyte with a detectable substance or an intermediate which can be reacted in solution to form a detectable substance. Alternatively, the particles can be used to suppress or remove an interfering substance. The apparatus is an improvement in systems for sample detection comprising a continuous flow sample source such as a process liquid source, carrier liquid-injected sample source sample separator column such as an ion exchange column or chromatographic column, or a capillary zone electrophoresis sample separator. It also includes a detector for detecting dissolved sample species, and connecting means including a flow passageway communicating with the sample source and detector means. The improvement comprises a particulate reagent reservoir for reagent particles having a particle size of less then 2 microns, and flow control means communicating with the particulate reagent reservoir and with the connecting means flow passageway for metering particulate reagent flow into the flow passageway. The detector should be capable of detecting dissolved sample species in the presence of a particulate reagent.

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Some reactions of the stream with the packed bed reactor may form precipitates. These precipitates may cause high resistance to stream flow or even plug the reactor and make the reactor unusable

Packed bed reactors cannot be used continuously. After bed depletion, the stream treatment process must be interrupted, and then the reactor is either regenerated or replaced.

Membrane reactions involve passage of the analyte stream across a membrane surface, and this is most efficiently achieved in flow detection systems by passing the stream through a tubular membrane or across a sheet membrane. The length of tubing or configuration of the sheet required to achieve a desired membrane reaction causes a further increase in dispersion, however, limiting the usefulness of this procedure. Membrane reactors are fragile and can burst easily. Also, the membrane is easily plugged or fouled with substances that cannot be removed and make the membrane unusable.

U.S. Patent No. 4,097,338 describes a method for determining a reduced coenzyme wherein the fluorescence of the reduced coenzyme is measured in an aqueous medium in the presence simultaneously of an organic liquid miscible with water and a dispersion of one or more slightly soluble or insoluble substances. The presence and combination of the organic liquid and particles enhances the fluorescence of the reduced coenzyme.

U.S. Patent No. 4,650,770 describes an immunoassay employing fluorescent particles and absorbent particles, wherein the absorbent particles substantially inhibit fluorescence when bound to the fluorescent particles through specific non-covalent binding. The fluorescence of the insoluble particles is measured.

West German Patent No. DE 2749956 describes an immunoassay using a photometric method of detection with latex polymer reagents. This is a kinetic method which cannot be readily adapted to flowing streams. Japanese Patents Nos. 59171863, 62002163, 62093663, and 62093664 are also directed to kinetic methods, a reading versus time end point with a batch solution, and cannot be readily adapted to flowing stream measurements. Instrument readings of the sample after substantial or complete reaction are generally required. In general, kinetic immunoassays are not useful in flowing stream detection methods of this invention because the Kinetic immunological reactions and reactions producing the detectable species are too slow and too specific.

U.S. Patent 4,665,020 describes a flow cytometer measurement of a binding competition immunoassay wherein a liquid sample containing analyte is mixed with reagent antigen coated fluorescent microspheres and larger microspheres coated with an antibody which binds specifically with the antigen. The particle suspension is measured by laser flow cytometer for fluorescent events and light scattering to provide data correlating to the analyte concentration in the sample. This is a specific immunoassay and no chromatographic separation is present. The insoluble fluorescent particles are measured.

East German Patent No. DD 219,873 describes a continuous flow method for determining HF and FeF_2/FeF_3 wherein an aqueous suspension of MgO is added to the sample. No chromatographic separation is involved, and this method cannot be extended to a chromatographic separation or other stream methods. The reagent is specific for only one compound in the mixture. Furthermore, the MgO is not insoluble, dissolving in the solution during the method.

Canadian Patent No. 1,103,137 describes a titration of an ion exchange colloidal polymer with an oppositely charged colloidal polymer.

Russian Patent No. SU 1,271,561 describes a counterflow addition of an ion exchange slurry in an industrial separation process. No detection of the slurry mixture is involved.

None of the above detection methods use insoluble particles to produce solutions of soluble materials to be detected. The analytical methods generally involve the production of particular particles correlating in concentration to a solution analyte, usually involving insolubilizing analyte, and measurement or determination of the particle products of the process. Other prior art methods used particles to initiate immunological or chemical reactions, and require completion of lengthy reactions before solution analysis can be performed; they cannot be readily adapted to the treatment and detection of a flowing stream. All of the above methods are specific and cannot be adapted to chromatographic methods.

A surface-enhansed Raman spectroscopy technique For HPLC and FIA detection was described by Freeman et al, *Applied Spectroscopy* 42:456 (1988). This method involves the production of silver particles correlating in concentration to a solution analyte, resulting in the insolubilization of the analyte and the measurement of the particle products of the process.

The object of this invention is to provide an improved apparatus and method for the analytical detection and measurement of dissolved components of a flowing liquid stream characterized by the development of an improved reactor for stream treatment and detection. In particular, the objective of this invention is to provide a detection apparatus and method comprising treating a liquid stream using chemical reagents to which a detector does not respond to reduce detector noise and increase sensitivity.

A further object of this invention to provide a more versatile detection apparatus and method. For

BRIEF DESCRIPTION 0F THE DRAWINGS

Fig. 1 is a high performance liquid chromatography (HPLC) and ion chromatography (IC) schematic representation of the apparatus of this invention:

Fig. 2 is a flow injection analysis (FIA) schematic representation of the apparatus of this invention.

Fig. 3 is a capillary zone electrophoresis (CZE) schematic representation of the apparatus of this invention.

Fig. 4 shows the effect of adding particulate reagent to a carbonate/bicarbonate eluant described in Example 1.

Fig. 5 and Fig. 6 are chromatograms described in Example 1.

Fig. 7 shows the effect of adding particulate reagent to a hydroxide eluant gradient described in Example 3.

Fig. 8 shows the effect of adding particulate reagent of a chloride eluant described in Example 10.

This invention is an analytical apparatus and method for determining the presence or concentration of original analyte in a solution. The method comprises an essential step of passing a solution containing a detectable substance through a detecting means for determining the presence of concentration of detectable substance in the solution. The improvement of this invention comprises mixing the solution with particulate reagent to form a suspension, the particulate reagent modifying the solution to yield a concentration of total detectable dissolved substance which correlates with the concentration of original analyte; and passing the solution through the detecting means which determines the presence and/or concentration of the dissolved detectable substance in the solution. This method is not a binding pair assay such as an immunoassay wherein reagent particles are modified by interaction with analyte in solution to effect a change in the reagent particles, and wherein the particle characteristics then are measured. The apparatus is an apparatus in which this method can be effected.

The apparatus and method of this invention are particularly advantageous for post-column treatment of liquid chromatographic eluant streams prior to detection measurement. The automatic detection of sample peaks as they elute from a liquid chromatographic column is necessary for fast, high performance separations. Hence, high performance liquid chromatography (HPLC) is often characterized by the type of detectors which are used. The most common detector for liquid chromatography is the spectrophotometer. Many organic compounds absorb strongly at the UV wavelengths. However, many compounds cannot be detected by this detector. Thus, there have been a multitude of different types of detectors developed for HPLC including amperometric, conductometric, fluorometric and refractometric detectors and the like.

The need for sensitivity and sample selectivity has led to the development of post-column derivatization techniques. Several schemes have also been developed. The most simple post-column reactions involve only the addition of energy in the form of heat, irradiation or electrons. Most other post-column reactions involve the addition of substances such as color-forming reagents or enzymes to react with sample species in the eluant stream.

These reagents have been added to the post-column eluant in essentially three ways. One approach has used solid packed bed column reactors. The reactor contains a reagent that reacts with the sample species and makes the peaks detectable. As the reagent is used, the column reactor must be replaced or regenerated. Another method of post-column derivatization has involved adding reagent dissolved in a solvent using a mixing tee. This approach has limited usefulness because many derivatization require the use of insoluble reagents. Also, other chemicals (such as a counter ion) that may interfere with the detection process may be present in the derivatization reagent. The last method for post-column addition of a reagent uses a membrane such as those used in ion chromatography. The membrane allows the passage of selected reagents to the eluant stream. However, the membranes are fragile, have a limiting surface area, and can be used only for certain reactions.

This invention introduces an apparatus and a new post-column treatment which are particularly useful in liquid chromatography. Insoluble solids are added post-column to the eluant stream. The solids are dispersed in the eluant and treats the eluant to make the sample peaks detectable. Prior to this invention, addition of solids to liquid chromatographic streams has been avoided. Usually HPLC solvents are filtered to remove all particles over 0.2 microns to extend column life and prevent plugging of the HPLC tubing detector cells.

A schematic representation of the HPLC and IC apparatus of this invention is shown in Fig. 1. The eluent reservoir 2 is connected by tubing and metering pump 4 through sample injection valve 6 to the inlet of the separation column 8. The sample injection valve 6 has a sample inlet means such as a conduit 10.

anion by ion exchange. In a still further alternative, the double suspension treatment technique can be combined with a treatment with a particulate reagent which masks sample peaks so that minor components can be determined. A particulate silver-form cation exchanger can be used to mask chloride ions in a nitrite ion determination, for example. Also barium-form cation exchange particles can be used to mask sulfate ions in a sulfuric acid trace anion determination.

In cation exchange separations, the anions in an acidic eluant containing an acid substance such as hydrochloric acid can be suppressed by treatment with particulate anion exchange resin in hydroxide form. This is useful for separation of alkali metal ions, alkaline earth metal ions and amines. In a double suspension treatment application of the invention, the eluant anion suppression is combined with treatment with a second particulate reagent which converts the analyte species into a common species to provide a uniform molar response in the detection step. This can be achieved, for example, by mixing the suspension with a particulate cation exchange resin which replaces the cationic analyte ions with a common cation by ion exchange. For transition metal containing eluants, lead ions in the eluant can be suppressed by treatment with a sulfate-form anion exchange resin slurry. The sulfate anions in sulfuric acid eluants can be suppressed by treatment with a lead-form or barium-form cation exchange resin slurry. In a still further alternative, the double suspension treatment technique can be combined with a treatment with a particulate reagent which masks sample peaks so that minor components can be determined. For example, the eluant can be treated with a chelating ion exchange resin slurry to remove transition metals in alkaline earth metal ion determinations.

In ion exclusion procedures, the method of this invention can be used to suppress the chloride ion in hydrochloric acid eluants with a silver-form cation exchange resin slurry. A weak organic acid eluant can be suppressed with a large-ammonium cation-form cation exchange resin slurry.

In mobile phase ion chromatography, a quaternary ammonium hydroxide eluant can be suppressed with a hydrogen-form cation exchange resin slurry.

Removal of the particulate reagent is unnecessary for many ion chromatography detectors such as amperometric, coulometric, potentiometric, fluorescence, and reflectance spectrophotometric detectors, although combination slurries may be required to suppress or remove eluant ions or convert a sample analyte to a detectable form.

In some instances, removal of particulate reagents may be necessary for spectrophotometric (UV-VIS), atomic emission and atomic absorption detectors, for example.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

HPLC methods, reagents, apparatus, detection systems, and post-column treatments are described by Krull (editor) in REACTION DETECTION IN LIQUID CHROMATOGRAPHY. New York: Marcel Dekker (1986), the entire contents of which and the publications cited therein being hereby incorporated by reference.

Applying the method of this invention, organic solvent eluant can be removed by particulate adsorbates such as the silicate material absorbent SILICALITE®. (Union Carbide). Particulates supporting chemical reactants and enzymes can be used for chemical reaction of the sample, for example enzyme oxidation or reduction. Ion pairing reagents can be removed by the procedures described above with regard to ion chromatography for masking peaks.

As with the ion chromatography detectors, removal of the particulate reagent is unnecessary for many HPLC detectors such as amperometric, coulometric, potentiometric, fluorescence, and reflectance spectrophotometric detectors. In some instances, removal of particulate reagents may be necessary for spectrophotometric (UV-VIS), atomic emission and atomic absorption detectors, for example.

CONTINUOUS FLOW ANALYSIS, FLOW INJECTION ANALYSIS, AND PROCESS MONITORS

These methods also involve the flow of a sample stream through a detector. Continuous flow analysis with segmented steams and flow injection analysis are described together with the equipment, reagents and detectors therefor by Ruzicka et al in FLOW INJECTION ANALYSIS. New York: John Wiley (1981), the entire contents of which and the references cited therein being hereby incorporated by reference. Continuous flow analysis and flow injection analysis involve the injection of a sample liquid in a flowing

charged, neutral, and negatively charged species at one end of the capillary tubing.

A regulated direct current high voltage power supply provides up to 30 kV potential. These voltages generate microampere currents through the capillary tube. Heat generated by the technique is dissipated through the capillary wall.

Fused silica is most commonly used for electrophoretic capillaries, although PTFE and polyethylene have also been used. Inner diameters of 50-100 microns, with a wall thickness of less than 200 microns are used in most applications. Capillary lengths of 50-100 cm are most frequently used.

Review articles on CZE have been written by Ewing et al, *Anal. Chem.* 61:292A (1989) and by Gordon et al, *Science*. 242: 224 (1988).

A scematic representation of the CZE apparatus of this invention is shown in Fig. 3. The buffer reservoir 72 is connected by tubing 74 through sample injection device 76 to the inlet of the separation capillary 78. The sample injection device 76 has a sample inlet means such as a conduit 10. The outlet of the separation capillary 78 is connected to a sheath 82. Buffer reservoir 72 and sheath 82 are in electrical contact with a high voltage source 84 which causes sample ions to migrate down separation capillary 78. Electrical contact of sheath 82 and separation capillary 78 is maintained with buffer pumped from buffer reservoir 86. The outlet 83 of sheath 82 is connected to a detector such as a conductivity cell 88 by conduit 90. The detector is connected with a conventional monitor 92 and recorder device 94.

The particulate reagent reservoirs can be used to introduce one or more particulate reagents into the liquid stream flowing through conduit 90. Reagent reservoirs 96 and 98 are unpressurized containers. Reservoir 96 is equipped with a stirrer 100 which maintains non-colloidal particles in suspension. Flow control from these reservoirs is maintained by metering pumps 102 and 104 which can be peristaltic pumps, for example. Reagent reservoir 106 is a pressurized container equipped with a gas inlet 108 for pressurized gas and a metering valve 110 to control liquid flow to the conduit 90. Other details and modifications of the apparatus will become apparent from the description provided hereinafter.

A solid reagent is added as a slurry or suspension post-column to the liquid stream 90 and dispersed in the stream to make a sample/solid flowing suspension. To avoid obstruction of the conduit 90, the conduit diameter should be at least 10 times the diameter of the largest particles of the particulate reagent. The solid treats the eluant to make the sample peaks detectable by either adding a component to the stream, extracting a component from the stream, or by performing both functions sequentially or simultaneously. The treatment may be applied to the entire eluant or to liquid segments containing concentrated analyte. However, because the treatment reagent is an insoluble solid, it does not interfere significantly with many detection processes.

All of the detectors that work for HPLC, IC, and FIA methods can also be used for CZE. However CZE flow rates are usually significantly less than other techniques. CZE detector and tubing void volumes are adjusted accordingly.

NON-FLOWING STREAM SAMPLE PREPARATION

The methods of this invention are also useful for treating liquid samples which are not flowing streams, for example, standard batch analytical methods wherein a dissolved detectable species is determined with a standard analytical detector. In general, the particles used in the methods of this invention remain in suspension during the detection step and are used to effect the changes described above for the flowing stream methods. Specific applications include pH adjustment of a sample with an appropriate ion exchange particle slurry; masking, for example with a chelating ion exchange resin slurry; and adsorption of contaminants, for example with a high surface area, non-polar solid to absorb organic components but not inorganic components of the solution. The detectors and special considerations required therewith are as described above with flowing stream methods.

PARTICULATE REAGENTS

Particulate reagents can be inorganic or organic. Inorganic reagents can be based on, for example, alumina, silica or molecular sieve materials. Organic reagents can be polymers, for example cross-linked forms of polystyrene, polyesters, polyamides, and cellulose. Depending on the application, the particulate reagent can be used directly for adsorption or absorption reactions or alternatively, the particulate reagent

TABLE 1

Titrant, mL	pH	MicroSiemens
0.00	3.90	2.50
2.00	4.00	2.55
4.00	4.10	2.55
6.00	4.20	2.45
8.00	4.40	2.30
9.00	4.50	2.20
10.00	4.70	2.10
10.50	4.90	1.95
11.00	5.10	1.90
11.40	5.40	1.90
11.60	5.60	2.00
11.80	6.00	2.05
12.00	6.50	2.25
12.20	8.80	2.55
12.40	9.20	2.85
12.60	9.40	3.20
12.80	9.50	3.45
13.00	9.55	3.70
13.20	9.60	5.10
13.40	9.65	6.50
13.60	9.70	8.40
13.80	9.75	12.2
14.00	9.80	17.8
14.50	9.85	27.2
15.00	9.95	36.5
16.00	10.10	53.5
17.00	10.20	71.6
18.00	10.30	88.3
19.00	10.40	104
20.00	10.40	119

The suspension reduced the conductivity of about 13 mL of NaOH titrant. As the titrate is added to the suspension, the conductivity of the suspension at first is reduced up to about 12 mL of titrant. This shows that the sulfonated particulate reagent has a low background conductance that is further reduced as the reagent is converted to the lower conducting sodium form.

This invention is further illustrated by the following specific but non-limiting examples. Temperatures are given in degrees centigrade and concentrations as weight percents unless otherwise specified. Procedures which are constructively reduced to practice herein are described in the present tense, and procedures which have been carried out in the laboratory are set forth in the past tense.

EXAMPLE 1

Ion Chromatography

This example demonstrates a method suitable for separating and quantifying several inorganic anions, specifically fluoride, chloride, nitrite, bromide, nitrate, phosphate, and sulfate, at concentrations, for example, ranging from 1 to 50 ppm. The aqueous mobile phase was a mixture of 0.0028 M $NaHCO_3$ and 0.0022 M Na_2CO_3. The flow rate was 1 mL/min, and the chart recorder rate was 2 min/cm.

The sample mixture was applied to a chromatographic separation column (15 cm x 4 mm) packed with

to successfully perform a eluant gradient, the background signal must remain as constant as possible.

A gradient of 5 mM to 50 mM KOH (carbonate not removed) was performed as a step gradient. The eluant (1 mL/min) was mixed with a 1 mL/min flow of a 2% suspension of less than 1 micron fully sulfonated gel-type cation exchanger (Benson Polymeric, Reno, NV). The detector was a WATERS MODEL 430 (Millipore, Corp., Milford, MA).

Fig. 7 shows the conductance of the eluant stream in the step gradient. A change of only 4 microSiemens conductance was measured. Without the addition of the particulate reagent, the conductance would be 85 microSiemens for the 5mM KOH and increased to 850 microSiemens as the eluant concentration is increased to 50mM KOH.

EXAMPLE 4

Ion Chromatography

This example describes the separation and quantification of several strong-acid sample anions - chloride, nitrate and sulfate -in the presence of a weak-acid sample anion, acetate.

The eluant is an aqueous solution of 0.020 M phthalic acid having a flow rate of 3.5 mL/min, and the column (10 cm x 4.6 mm) is a low-capacity anion exchanger (Cat. No. 269013, Wescan Instruments).

After chromatographic separation of the sample, the eluant is directed to a mixing tee where it is mixed with a 5% suspension of 0.2 micron, high surface area, macroporous polymer (P 80, poly(styrene-divinyl benzene), Benson Polymeric, Reno, NV). The particulate suspension reagent flow is 1 ml/min. The suspension mixture of column eluant and reagent is directed to the cell of a conductivity reactor (Model 213A, Wescan Instruments). The low pH eluant elutes acetate with the solvent peak, and chloride, nitrate, and sulfate, respectively, are eluted in an 8 minute separation. The suspension reagent adsorbs the non-polar eluant reagent, phthalic acid, from the aqueous solution but does not affect the sample anions which are in the form of highly conducting acetic, hydrochloric, nitric and sulfuric acids.

EXAMPLE 5

Ion Chromatography

This example uses electrochemical amperometric detection. Particulate reagent is used to absorb organic compounds in the sample that might deactivate or poison the working electrode of the detector.

A 10 cm x 7.8 mm anion exclusion column (high capacity, sulfonated, 8% cross-linked, poly(styrene-divinylbenzene) resin, Benson Polymeric, Reno, NV) is used to separate sulfite in beer. Beer contains organics that may adsorb on the working electrode of a detector. The eluant is aqueous 0.005 M sulfuric acid set to a flow rate of 0.85 mL/min. The sulfite sample is injected, and sulfite elutes at about 3.5 min. The column effluent is directed to a mixing chamber where it is mixed with a 1% suspension of 0.1 micron, high surface area (415 M^2/g), macroporous, poly(styrene-divinylbenzene) resin. Then, the stream is directed to an amperometric detector (Model 271, Wescan Instruments) with a Pt electrode adjusted to +0.6 volts. The particulate reagent adsorbs organics that elute from the column, but does not affect the oxidation and detection of the sulfite ions.

EXAMPLE 6

Ion Chromatography

EXAMPLE 9

Ion Chromatography

In this example, ion chromatography is used to separate transition metals. The hydroxide suppressor particulate reagent cannot be used for these metal cations, as it is used for the alkali metals of Example 4, because hydroxide precipitates some transition metals and thus prevents them from being detected.

Copper(II), nickel(II), zinc(II), and cadmium(II) are separated on a 15 cm x 4.0 mm low capacity, cation exchange column (Benson Polymeric, Reno, NV). The mobile phase is 0.005 M $BaCl_2$ at a flow rate of 1.5 mL min. The sample is injected, and the metal ions eluted in the order: copper, nickel, zinc, and cadmium. As the metals elute from the column, the column effluent is directed to a mixing tee where it is mixed with a 1% suspension of 0.1 micron high capacity, strong base, cation exchange resin in the sulfate form. The particulate reagent suspension stream flow is 1 ml/min. After reaction, the stream is directed to a conductivity detector (Model 213A, Wescan Instruments).

The suspension reagent converts the eluant to low conducting water by precipitating the barium cation with the sulfate on the reagent. Metal cations are detected as the metal sulfates.

EXAMPLE 10

Ion Chromatography

Low molecular weight organic acids are separated by ion exclusion chromatography. In this example, oxalic, maleic, malic, succinic, formic and acetic acids are separated on a glass lined column (30 cm x 7.8 mm) packed with a fully sulfonated styrene-divinylbenzene copolymer (Polymeric resin Cat. No. 825, Benson Polymeric, Reno, NV). The eluant is 0.003 N HCl at a flow rate of 0.7 mL/min. After the separation of the organic acids, the eluant is directed to a mixing tee where it is mixed with a suspension of 0.2 micron high capacity cation exchange resin in the silver form. The suspension stream flow is 1 mL/min. The mixture is directed through the conductivity cell of a conductivity detector (Model 213A, Wescan Instruments). The suspension converts the eluant to low conducting water by precipitating the chloride anion with the silver cation on the particles. The suspension does not affect the organic acid analytes. The separation is completed in less than 12 min with the sample peaks eluting in the order oxalic, maleic, malic, succinic, formic and acetic acids.

An example of precipitation of a chloride containing eluant with a Ag-form cation exchange less than 1 micron particulate suspension was performed. A stream of 10 mM NaCl was treated with a 2% suspension. Fig. 8 shows the conductance without treatment was 314 microSiemens (Model 213A, Wescan Instruments). After treatment, the stream conductance decreased to 2.9 microSiemens.

EXAMPLE 11

HPLC

In this example, HPLC is used to separate and detect anionic surfactants, i.e., alkyl benzene sulfonates. The mobile phase is made with 0.01 N $(NH_4)_3B_{10}O_{16}\cdot 8H_2O$ (ammonium borate) and 0.01 M boric acid to produce an aqueous mobile phase having a pH of 8.3. The retention of anionic surfactant is controlled by varying acetonitrile concentration in the mobile phase while retaining the boric acid and ammonium borate levels at constant concentrations Gradient elution is used with the acetonitrile concentration varied from 50% to 90%. The flow rate is 1.5 mL/min. The HPLC reverse phase column is (15 cm x 4.6 mm) is packed

bases in the presence of low conducting organic base.

Claims

1. An analytical method for determining the presence or concentration of original analyte in a solution comprising detecting a detectable dissolved substance in solution with a detecting means for determining the presence or concentration of the dissolved substance, characterized by

a) mixing the solution with particulate reagent to form a suspension, the particulate reagent modifying the solution to yield a concentration of total detectable substance which correlates with the concentration of original analyte while remaining insoluble, wherein the particulate reagent does not have bound thereto, an antibody, antibody binding fragment, or antigen selected for specific antibody binding reactions; and

b) detecting th presence or concentration of the detectable dissolved substance in the solution with the detecting means.

2. The method of Claim 1 for determining the presence or concentration or original analyte in a solution comprising passing a solution containing dissolved detectable substance through the detecting means, characterized by

a) mixing the solution with particulate reagent to form a suspension, the particulate reagent modifying the solution to yield a concentration of total solubilized detectable substance which correlates with the concentration of original analyte; and

b) passing the solution through the detecting means.

3. The method of Claim 2 wherein the particulate reagent interacts with the analyte to yield a corresponding concentration of total detectable substance in the solution.

4. The method of one or more of the Claims 1 - 3 wherein the particulate reagent comprises dispersible ion exchange particles having an exchangeable substance thereon which is displaced by the analyte for a second substance, e.g. a solubilized detectable substance, the concentration of the second substance correlating with the concentration of analyte originally in the solution.

5. The method of Claim 4 wherein the second substance reacts with reagent in the solution to provide a detectable dissolved substance in a concentration which correlates with the concentration of analyte.

6. The method of one or more of the Claims 1 - 5 wherein the particulate reagent comprises particles which chemically or enzymatically react with the analyte to yield a reaction product which is either a detectable substance or which can be converted by reaction with dissolved or particulate reagent in the solution to provide a detectable substance.

7. The method of one or more of the Claims 1 - 6 wherein the solution contains an interfering substance and the particulate reagent interacts with the interfering substance to reduce its level in the solution by ion exchange, chelation, chemical reaction, enzymatic reaction, adsorption, or absorption.

8. The method of one or more of the Claims 1 - 7, wherein the particulate reagent remains in the solution passing through the detecting means.

9. The method of one or more of the Claims 1 - 8, wherein the particulate reagent is an ion exchange material, chemical reactant, adsorbent, absorbent, particles having enzymes bound thereto, or particles having binding partners for interfering substances bound thereto.

10. The method of one or more of the Claims 1 - 9, wherein the analyte solution is mixed with a plurality of particulate reagents.

11. An apparatus for sample detection comprising a continuous flow sample inlet, a detector means for detecting dissolved sample species, and connecting means including a flow passageway communicating with the continuous flow sample inlet and detector means, characterized by a particulate reagent reservoir means for reagent particles having a particle size of less then 2 microns, and flow control means, which may include a flow control valve, communicating with the particulate reagent reservoir and with the connecting means flow passageway for metering particulate reagent flow into the flow passageway, the detector means being a means for detecting dissolved sample species in the presence of a particulate reagent.

12. An apparatus of claim 11 wherein the detector is a conductivity meter having electrodes which will not be bridged or obstructed by particulate reagent, e.g. the electrodes being without pores having a diameter less than 10 times the diameter of the largest particulate reagent particles and the electrodes having a spacing which is at least 2 times the diameter of said particle.

FIG._1

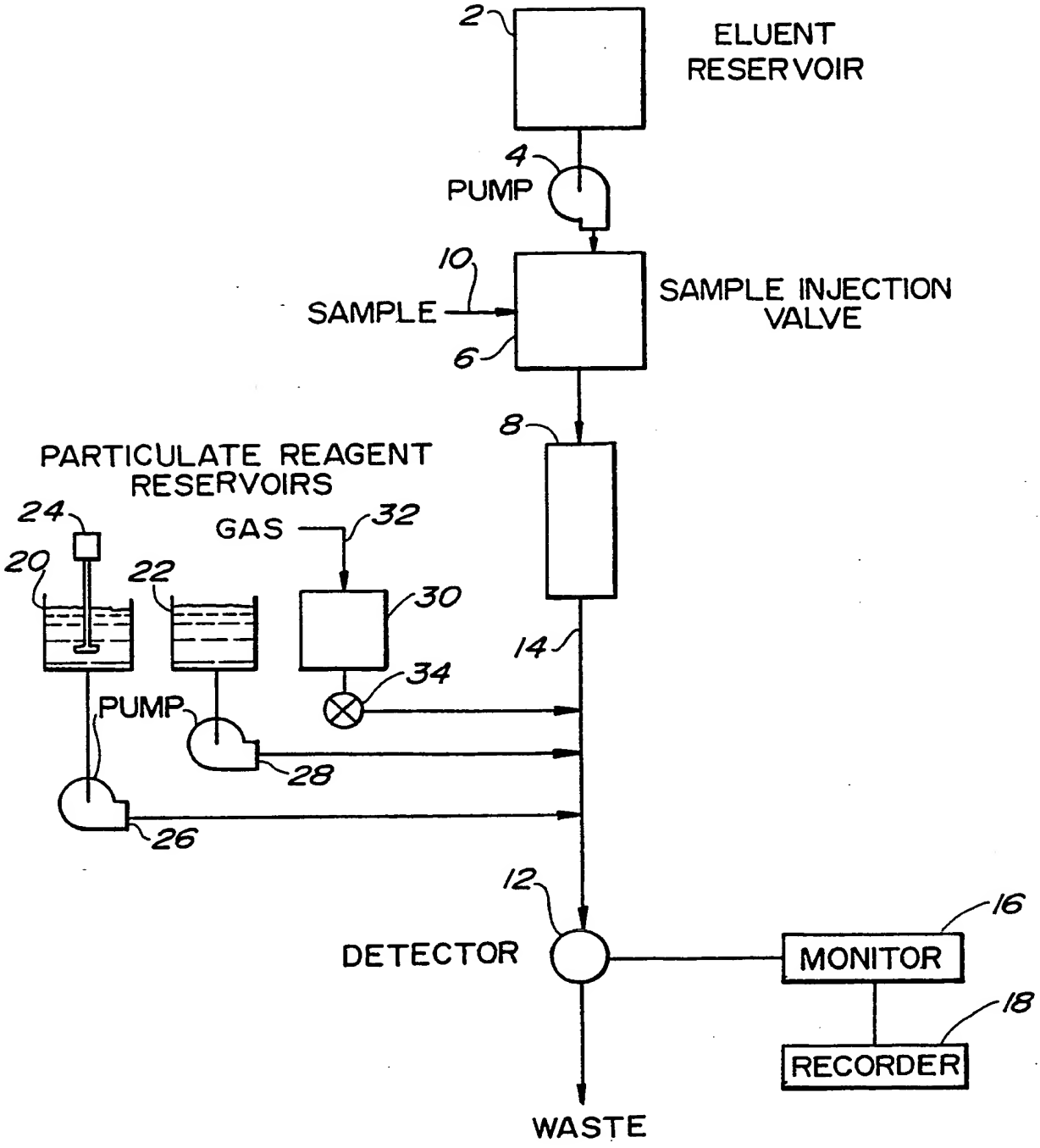
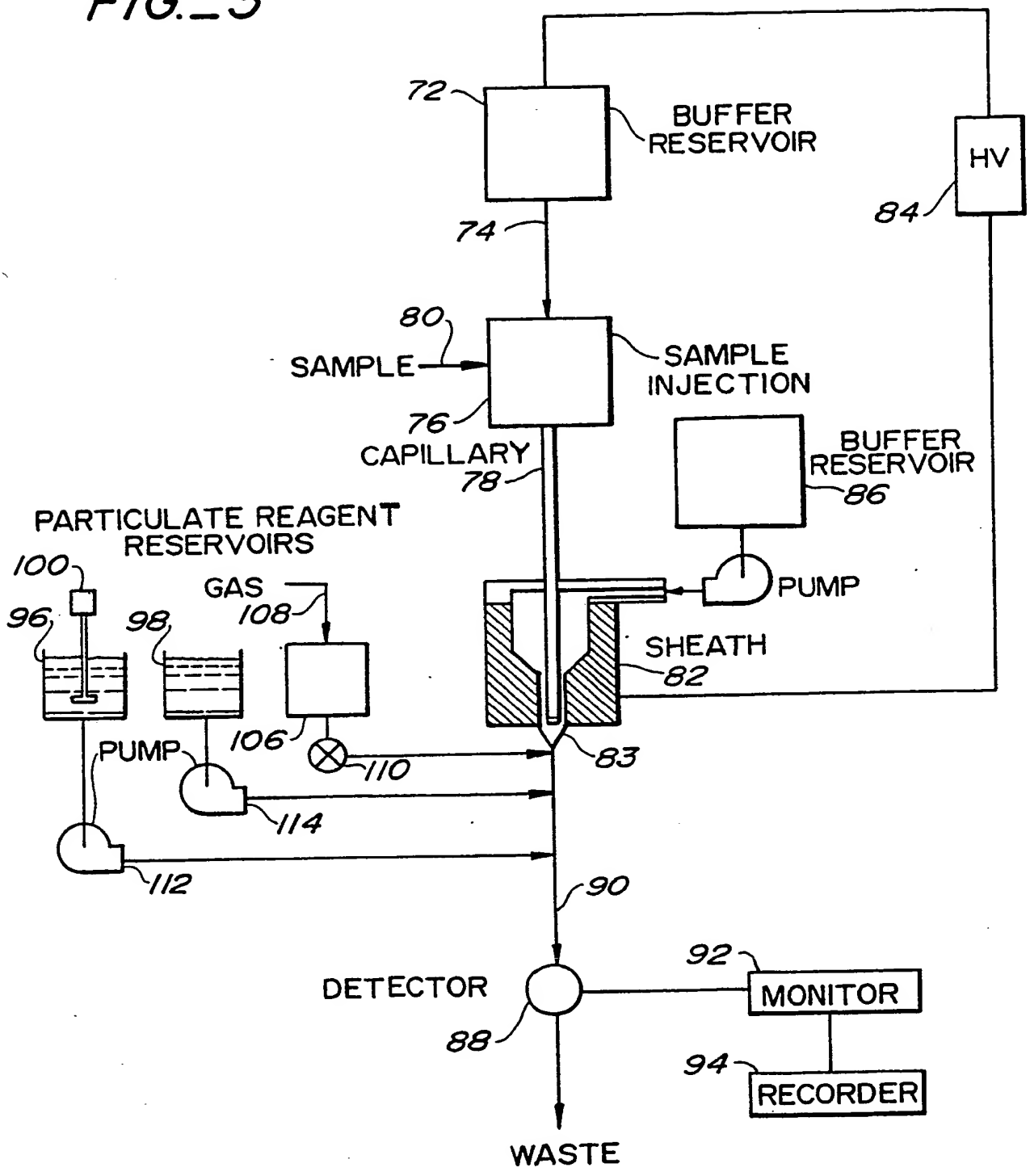

2
ELUENT RESERVOIR
4
PUMP
10
SAMPLE
6
SAMPLE INJECTION VALVE
8
PARTICULATE REAGENT RESERVOIRS
24
GAS
32
20
22
30
14
34
PUMP
28
26
12
DETECTOR
16
MONITOR
18
RECORDER
WASTE

FIG._3


72
BUFFER RESERVOIR
HV
84
74
80
SAMPLE
SAMPLE INJECTION
76
CAPILLARY
78
BUFFER RESERVOIR
86
PARTICULATE REAGENT RESERVOIRS
100
96
98
GAS
108
PUMP
SHEATH
82
106
110
83
PUMP
114
112
90
92
DETECTOR
MONITOR
88
94
RECORDER
WASTE

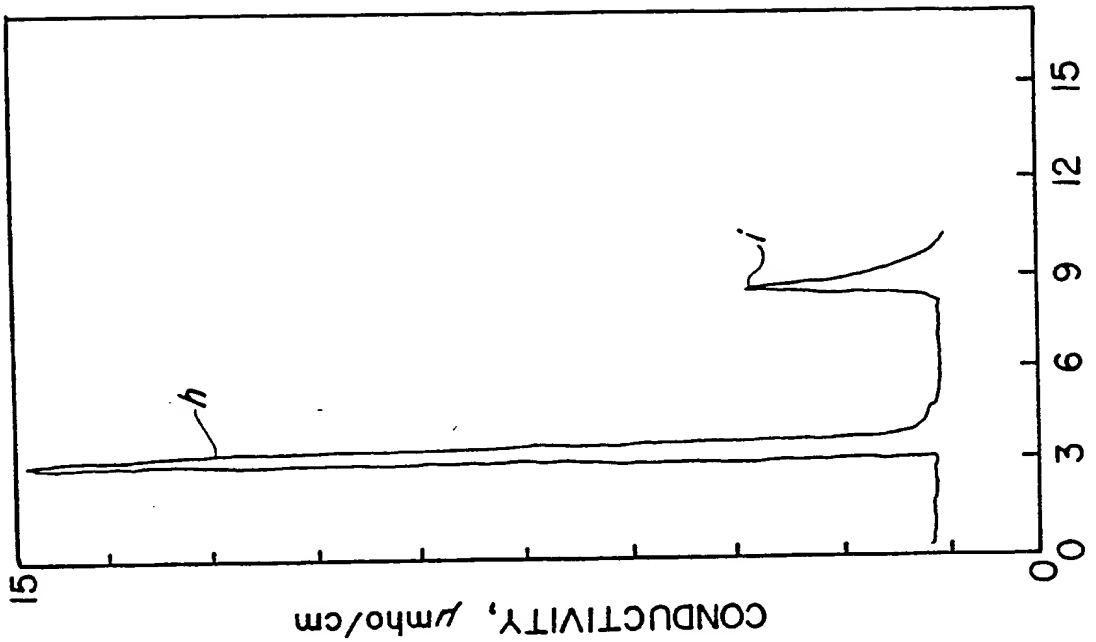


FIG._6.

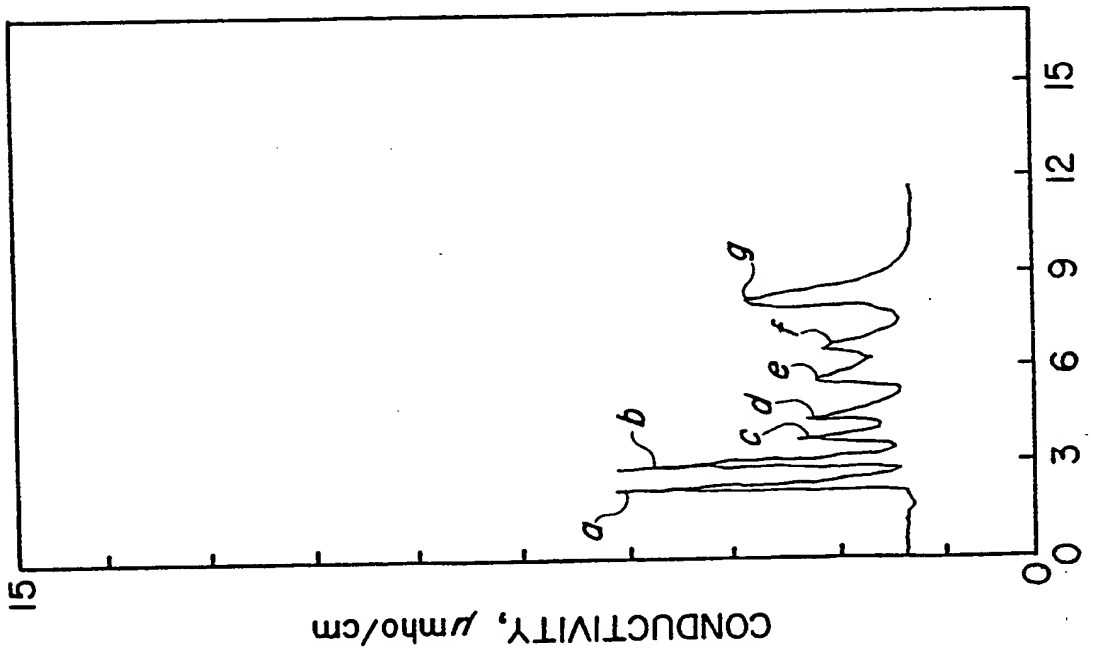


FIG._5.

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(54) Fluid analysis with particulate reagent suspension.

(57) An improvement in the analytical method for determining the presence or concentration of analyte in a solution comprising detecting a dissolved detectable substance in solution with a detecting means, for example, by passing a solution containing a detectable substance through a detection means for determining the presence of concentration of detectable substance in the solution. The improvement comprises mixing the solution with one or more particulate reagents to form a suspension, the particulate reagents modifying the solution to yield a concentration of total detectable substance which correlates with the concentration of original analyte, and passing the solution through the detecting means. The particles can be used to replace an analyte with a detectable substance or an intermediate which can be reacted in solution to form a detectable substance. Alternatively, the particles can be used to suppress or remove an interfering substance. The apparatus is an improvement in systems for sample detection comprising a continuous flow sample source such as a process liquid source, carrier liquid-injected sample source sample separator column such as an ion exchange column or chromatographic column, or a capillary zone electrophoresis sample separator. It also includes a detector for detecting dissolved sample species, and connecting means including a flow passageway communicating with the sample source and detector means. The improvement comprises a particulate reagent reservoir for reagent particles having a particle size of less then 2 microns, and flow control means communicating with the particulate reagent reservoir and with the connecting means flow passageway for metering particulate reagent flow into the flow passageway. The detector should be capable of detecting dissolved sample species in the presence of a particulate reagent.

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